REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.
PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED
01-11-2004	Final Report	Jun 2000 - May 2004

4. TITLE AND SUBTITLE

Engineering Analysis of a Genetic Switch: Modeling and Simulation Tools

5a. CONTRACT NUMBER

5b. GRANT NUMBER

N00014-00-1-0758

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Arkin, Adam, P

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Berkeley
mailcode 5230
Calvin Lab
Berkeley, CA 94720-5230

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 N. Quincy Street
Arlington, CA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)

ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution Unlimited

20041112 025

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall objective of this project was quantitatively explain the extraordinary stability of the λ-phage lysogen given that the data on the lysis/lysogeny switch dynamics didn't seem to be sufficient for explanation. Further, we aimed to explain the differential stability of a number of operator mutants constructed by John Little. Further, in all cases, we wished to explain the threshold response of the induction switch to different exposures to UV light. Then, based on this understanding we aimed to re-engineer this switch with different thresholds, cooperativities and stabilities.

15. SUBJECT TERMS

lambda phage, lysogen, switch

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Adam P. Arkin
unclass	unclass	unclass	UL	Self Expl	19b. TELEPHONE NUMBER *(Include area code)* 510-495-2366

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

Objective: The overall objective of this project was
quantitatively explain the extraordinary stability of the λ-
phage lysogen given that the data on the lysis/lysogeny
switch dynamics didn't seem to be sufficient for
explanation. Further, we aimed to explain the differential
stability of a number of operator mutants constructed by
John Little. Further, in all cases, we wished to explain
the threshold response of the induction switch to different
exposures to UV light. Then, based on this understanding
we aimed to re-engineer this switch with different
thresholds, cooperativities and stabilities.

Approach: John Little made a number of directed mutants
that changed the predicted feedback structure, binding
affinities/cooperativities, and promoter activities of the
lambda lysogen. Robin Osterhout from the Arkin lab interned
with John Little and worked with him here during his
sabbatical to learn the UV induction experiments and with
Michael Laub at the CGR at Harvard and Jay Keasling here at
Berkeley to learn the microarray analysis we would use to
follow host-phage response. She collected (and is still
collecting) time-series data on gene expression of each
mutant under different UV conditions. These data, then,
form a multidimensional matrix with genes on one axis, λ-
mutant on the other, experiment on another and time on the
last. Robin and Sergey Plyasunov from the Arkin lab have
collaborated with little in developing new models of the
induction dynamics. First, we have broken everything in the
system into elementary reactions and introduced a new
biophysical model of the newly discovered PR/PL looping
regulation dynamics. This has allowed us to perform a full
stochastic sensitivity analysis of the network to deduce
what the key controlling factors are. Transcription factor
OFF rates and the looping ON rate are the most important
features. We have also adapted an extended a method called
transition path sampling used in statistical mechanics to
application of the chemical master equation which allows us
to accurately estimate the transition probabilities between
different stable states of our chemical system. This has
allowed us to use our model to calculate the stability of

the lysogen even though spontaneous inductions rates are less the 1 in 10^9.

Accomplishments: We have built detailed models of the induction switch including major revisions to the kinetics of the lambda right promoter and a new model of DNA looping. We have developed a new methodology for accurately estimating the frequency of low-probability events. We have used to model to make predictions about the sensitivity of the lambda switch to different mutations. Experimentally, we have recently gotten the microarray analysis for the E.coli/λ system working sufficiently well to provide the data necessary for testing the model. Wild-type and four different λ mutants have been exposed to differing dosages of UV and a time courses of their response have been collected. The gene expression during this response is being followed now by microarray.

Conclusions: Such quantitative study of even so simple a genetic switch requires the very careful and rigorous application of a number of difficult technologies. While we did not accomplish all our goals within the project period, this grant allowed us to get the pipeline up and running. Now with new simulation technology, new biophysical models, and new experimental techniques in hand, we are in a position to answer all the questions we started out with. Our initial results indicate that the newly discovered PR/PL looping regulation is sufficient to explain all of Prof. Little's mutant results but that there are difficulties in explaining the incredible stability of wild-type. However, we await the full data matrix to compare our models to before we make any final conclusions.

Significance: Apart from developing and hardening a entirely new technology for screening the activity of new molecules and identifying their protein targets—this method is now being adapted to screen for new classes of druggable proteins for use in human therapeutics and for deducing networks of interaction in the yeast cell.

Patent Information: No patents have been filed related to this research

Award Information: 2001, Arkin Elected to the Howard Hughes Medical Institute

Publication and Abstracts:

Two papers are in preparation on the modeling technology developed for this grant—but further publication awaits collection of the data.